

Retinoblastoma, the visible CNS tumor: a review

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ABSTRACT

The pediatric ocular cancer retinoblastoma is the only central nervous system tumor readily observed without specialized equipment: it can be seen by, and in, the naked eye. This accessibility enables unique imaging modalities. Here, we review this cancer for a neuroscience audience, highlighting these clinical and research imaging options, including fundus imaging, optical coherence tomography, ultrasound, and magnetic resonance imaging. We also discuss the subtype of retinoblastoma driven by the *MYCN* oncogene more commonly associated with neuroblastoma, and consider trilateral retinoblastoma, in which an intracranial tumor arises along with ocular tumors in patients with germline *RB1* gene mutations. Retinoblastoma research and clinical care can offer insights applicable to CNS malignancies, and also benefit from approaches developed elsewhere in the CNS.

SIGNIFICANCE

Retinoblastoma is the most common eye cancer in children. It arises in the developing retina, part of the central nervous system (CNS). Thus, retinoblastoma is a CNS cancer viewable from outside. Retinoblastoma is usually caused by mutations in the *RB1* gene, but can also be caused by amplification of the neuroblastoma oncogene *MYCN*. Rarely, *RB1* gene mutations cause a tumor within the brain as well as eye tumors. Retinoblastoma can be imaged by multiple means, including visually through the eye, by ultrasound, magnetic resonance, and optical coherence tomography. Together, these features make retinoblastoma a unique case study amongst CNS tumors.

INTRODUCTION

The pediatric retinal tumor retinoblastoma is the most common eye cancer in children. It is unique amongst CNS tumors as it is visible through the eye without requiring invasive imaging. This provides an unparalleled opportunity to image tumors as they develop or respond to treatment. Moreover, retinoblastoma provides intriguing insights into nervous system disease in its trilateral and *MYCN*-driven forms.

In this review, we introduce retinoblastoma with a focus on these features. In the first part of the review, we describe the clinical, molecular and histological features of this tumor in the context of its treatment and prognosis. In the second section of the review, we focus on innovations in the imaging of retinoblastoma to highlight what this pediatric ocular cancer tells us about CNS malignancy. We follow this with a discussion of the aforementioned trilateral and *MYCN*-driven forms of retinoblastoma, with the goal of highlighting what retinoblastoma research can gain from studies of other CNS malignancies, and vice versa.

RETINOBLASTOMA OVERVIEW

Clinical vignette. A mother in urban Canada is taking photographs of her child, when she notices a strange white reflex in the child's eye (Fig. 1a). Unsure what it is, later that night she types "white eye in baby" into an Internet search engine. A list of results pops up, alerting her that a white pupil could be a sign of cancer. She immediately seeks attention at the nearest hospital, where the child is diagnosed with retinoblastoma.

Similarly, one evening a mother in rural India notices a white reflex in the eye of her child. Her baby does not seem bothered by it, so she does not worry about it too much. Later that month a community health worker arrives to check on the family. The mother asks about the odd white reflection in her baby's eye. The health worker has just been trained on a new app that helps in the diagnosis of eye conditions. Using her smartphone, she takes a flash photo of the child's eye, and uses the app's database of eye conditions to compare her photograph. She realizes the photo she took looks similar to the database photo of retinoblastoma, so she refers the family to hospital.

Clinical features. These hypothetical cases illustrate typical presentations of retinoblastoma. The incidence of retinoblastoma is constant worldwide at 1 in every 16,000 live births (Dimaras et al. 2015; Dimaras et al. 2012). As such, countries with higher birth rates and populations observe the highest number of cases. The most common first signs of retinoblastoma are leukocoria, a white reflex visible through the pupil (Fig. 1a), and strabismus, misaligned eyes. Awareness of these early signs of eye cancer is crucial to good outcomes for affected children. However, where awareness of these signs is low, and access to healthcare is challenging, retinoblastoma is often diagnosed late. In this case, the most common sign at presentation may be proptosis, where tumor causes the eye to bulge from the orbit. Retinoblastoma can affect one or both eyes, and sometimes also the pineal, parasellar or suprasellar regions (trilateral retinoblastoma, discussed below).

Detection of retinoblastoma by lay people is possible because retinoblastoma is a visible tumor with distinct features. Several awareness efforts aim to improve public knowledge of the early visible signs of retinoblastoma. These include low-tech

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3 innovations, such as including images of leukocoria in Maternal Child Health booklets
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5 (Ministry of Health Kenya 2010) provided to mothers shortly after childbirth, to alert
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7 them of the signs of retinoblastoma. High-tech innovations include concepts like the
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9 Portable Eye Examination Kit (Peek), a smartphone application that allows users to
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11 recognize and detect eye conditions, including retinoblastoma (Bastawrous 2016;
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13 Bastawrous et al. 2015; Lodhia et al. 2016; Morjaria et al. 2017).
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18 Medical diagnosis of retinoblastoma is based on the clinical features of the tumors
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20 visible in the eye upon dilation of the pupil (Dimaras et al. 2012) (Fig. 1b). This differs
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22 from diagnosis of other cancers, where histological confirmation via biopsy is usually
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24 required. Biopsy of retinoblastoma is not recommended, as it can induce seeding and
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26 extraocular spread along the needle tract (Karcioglu 2002).
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30 Retinoblastoma is often highly calcified (Fig. 1c) (Bullock et al. 1977). Calcification is
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32 visible to the naked eye, and can also be detected by ultrasound or MRI (Rodjan et al.
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34 2015) (see below). Calcification in retinoblastoma is 'dystrophic' (Eagle 2013), that is, it
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36 occurs in response to tissue damage or necrosis. It is more often observed in tumors of
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38 older children and advanced retinoblastoma (Levy et al. 2011).
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43 Retinoma, the benign precursor of retinoblastoma, also displays calcification, along
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45 with several distinct clinical features; it is described clinically as a grey, translucent
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47 mass, often with chorioretinal atrophy and variation in the retinal pigment epithelium
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49 (Dimaras et al. 2009; Dimaras et al. 2008; Gallie et al. 1982b). Retinoma is rare, but can
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51 be sometimes observed in parents of children with retinoblastoma (Dimaras et al. 2008;
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Gallie et al. 1982a). Its rare occurrence suggests that most retinomas undergo malignant transformation to retinoblastoma.

Growing retinoblastoma tumors produce seeds that detach and adhere below the retina (subretinal seeds) or float into the vitreous (vitreous seeds). The appearance of seeds has been described as fine dust, spheres, or clouds, each with a progressively worse prognosis, respectively (Francis et al. 2015; Francis et al. 2016; Munier 2014). Large tumors can lift and detach the retina, and cause significant inflammation and necrosis, sometimes leading to phthisis bulbi (shrinkage of the eye) (Gallie et al. 1982b; Kashyap et al. 2011).

Tumor can spread beyond the eye by surpassing retinal boundaries, such as Bruch’s membrane, to invade the choroid and reach the blood supply. Another common route for tumor dissemination is via the optic nerve to reach the brain and cerebrospinal fluid (CSF) (Mallipatna et al. 2017).

Staging. The features of retinoblastoma at presentation provide data to accurately classify the disease in each eye, and consequently direct treatment. During the era when retinoblastoma was primarily treated by radiation, the Reese-Ellsworth Classification (Reese and Ellsworth 1963) system was used, separating tumor classes by their predicted response to radiation treatment. As systemic chemotherapy began to replace radiation as the most common form of treatment, the International Intraocular Retinoblastoma Classification (IIRC) (Murphree 2005) system was developed. This system is largely based on natural progression of disease from small tumors away from the macula (easily treatable with laser therapy) and larger, central tumors with variable

degrees of seeding and retinal detachment (requiring chemotherapy in addition to focal therapy). Modifications to the original IIRC were later introduced by another group (Shields et al. 2006), making it difficult to compare across studies and centers (Novetsky et al. 2009). The critical differences between the classification systems have previously been reviewed (Dimaras et al. 2015). Other classification schemes include staging of extraocular retinoblastoma (Chantada et al. 2006) and staging of vitreous seeding (Francis et al. 2015; Francis et al. 2016; Munier 2014).

As with other cancers, retinoblastoma is also staged using the American Joint Commission on Cancer (AJCC)'s "Tumor Node Metastasis" (TNM) classification system. The most recent 8th edition of the TNM (Mallipatna et al. 2017) aims to provide a way forward for retinoblastoma classification given the above eye-based classification confusion. The Tumor stage is first applied per eye based on clinical features, and the patient's score is based on the most advanced eye (Mallipatna et al. 2017). Radiology plays an important role in determining the Metastasis stage, particularly in delineating any involvement of the CNS. The 8th edition of the TNM also includes a new feature, the additional "H" or heritable trait, which denotes risk of multifocal tumors that can affect both eyes and brain (trilateral retinoblastoma). As yet, trilateral retinoblastoma is not staged by the TNM system. In fact, no other tumors of the CNS are staged by TNM, as tumor size is not a prognostic indicator, the brain and spinal cord do not have a lymphatic system, and metastases are rarely distant (Laws Jr. et al. 2017). Retinoblastoma is a unique CNS tumor in this respect, as tumor size is prognostic, it can affect preauricular, submandibular or cervical nodes, and distant metastases occur (Mallipatna et al. 2017).

Pathology. Retinoblastoma is a small round blue-cell tumor (Fig. 1d-e), and thus bears histologic similarity to other childhood nervous system tumors including neuroblastoma and medulloblastoma. Cells are primarily composed of large basophilic nuclei and little cytoplasm. The tumor is highly proliferative, displaying abundant mitoses and necrosis. Cells may take on a polygonal shape and mimic cobblestones when packed together (Tso 1980). Often, collars of viable cells can be observed around a blood vessel, surrounded by necrosing cells (Fig. 1f). Tumors display varying degrees of differentiation. Cellular differentiation is primarily in the form of Flexner-Wintersteiner rosettes and Homer Wright rosettes (Tso 1980) (Fig. 1e). Flexner-Wintersteiner rosettes are composed of an ‘empty’ lumen surrounded by columnar cells (Wippold and Perry 2006). Homer Wright rosettes consist of cells surrounding a central lumen made up of their processes (Wippold and Perry 2006). While each type is individually common in other neural tumors, the dual presence of Homer Wright and Flexner-Wintersteiner rosettes is pathognomonic for retinoblastoma.

Retinoma histopathology is distinct from retinoblastoma (Fig. 1g). This benign precursor is composed of sparsely spaced round cells, and does not contain rosettes. Instead, it is characterized by the presence of fleurettes, composed of clusters of cells with long, bulbous cytoplasmic processes, mimicking the *fleur-de-lys* (Dimaras et al. 2009; Dimaras et al. 2008). Retinoma is observed next to retinoblastoma in 16-20% of eyes enucleated for retinoblastoma (Dimaras et al. 2008; Eagle 2009).

Cellular differentiation has not been conclusively linked to prognosis, and thus does not form part of the histological evaluation of tumors. However, the degree of anaplasia evident in retinoblastoma has been shown to correlate with prognosis (Mendoza et al.

2015), but this has not yet become part of routine histological evaluation. The pathology TNM (pTNM) classification determines extent of tumor involvement of the optic nerve, choroid, sclera and anterior segment (Finger 2009; Gallie et al. 2016; Mallipatna et al. 2017). High risk of metastasis (pT3) is conveyed by >3 mm of tumor in the choroid (the vascular layer of the eye, under the retina), tumor extending beyond the lamina cribrosa of the optic nerve (the perforated or 'mesh-like' structure in the sclera through which the fibers of the optic nerve exit the eye), or significant invasion of the sclera. Extraocular disease (pT4) is marked by tumor at the cut end of the nerve, involvement of the episclera, or invasion of adjacent bone, eyelid, conjunctiva, or muscle (Mallipatna et al. 2017).

Genetics. Retinoblastoma is perhaps best known to cancer biologists as the inspiration for the "two-hit hypothesis" for oncogenesis, which states that loss of both alleles of a tumor suppressor gene are necessary to initiate cancer. Working in the pre-genomic era, Alfred Knudson noted an earlier age of onset for bilateral retinoblastoma than for unilateral (Knudson 1971). His mathematical analysis implied two rate-limiting steps were needed for unilateral retinoblastoma to develop, while only a single rate-limiting step was required for bilateral cases. Fifteen years later, the "retinoblastoma gene," *RB1*, was cloned, confirming Knudson's prediction (Friend et al. 1986; Fung et al. 1987; Lee et al. 1987). Unilateral patients (usually) have lost both wild-type alleles of *RB1* in a susceptible retinal cell (two hits), leading to a single, unifocal tumor (Fig. 2). Conversely, all bilateral patients have a germline mutation in *RB1*, thus only require a second hit to initiate cancer, which (usually) happens in multiple cells, in both eyes. The situation is complicated somewhat by low-penetrance alleles, which can be mutated in

the germline without promoting bilateral disease (Lohmann et al. 1994). Because of this, approximately 15% of unilateral cases have a heritable mutation.

The irony is not lost on us that while being a visible tumor, retinoblastoma can rob vision from the children it affects. If patients grow up to have children of their own, they should learn if they have a heritable mutation that could affect their offspring. For all families with heritable retinoblastoma, the “visible feature” of retinoblastoma that allows early detection is knowledge of the *RB1* mutation. Prenatal genetic detection coupled with early term delivery (37-38 weeks’ gestation) of the affected infant allows clinical teams to monitor the newborn’s retina for the earliest sign of tumor and treat it immediately (Soliman et al. 2016).

The *RB1* gene encodes pRB, a key negative regulatory transcription factor governing the G₁ to S phase cell cycle transition by blocking the interactions of DNA-binding E2F and DP transcription factors with DNA (Dick and Rubin 2013). In response to mitogen-stimulated cyclin-dependent kinase signaling, normal pRB is hyperphosphorylated, releasing it from E2F interactions, allowing these factors to activate transcription. pRB also has a growing spectrum of roles in differentiation, chromatin remodeling, genome stability, and apoptosis (Dimaras and Corson 2016; Velez-Cruz and Johnson 2017).

Over 1000 mutations in *RB1* have been documented in retinoblastoma tumors, including missense and nonsense mutations, splicing mutations, frameshifts, micro- and macrodeletions, and promoter methylation (Lohmann and Gallie 2004). Despite this, a small fraction of unilateral cases lack identified *RB1* mutations, so it was long postulated

that an alternative mechanism of retinoblastomagenesis might be possible. As part of an international team, in 2013 we identified that 1.4% of unilateral retinoblastoma, lacking *RB1* mutations, instead showed amplification of *MYCN* (Rushlow et al. 2013) (Fig. 2), a transcription factor-encoding gene more frequently associated with the pediatric peripheral nervous system tumor, neuroblastoma (Ruiz-Perez et al. 2017). This finding raises interesting questions about the parallels between retinoblastoma and other neuronal tumors; see below.

Even in retinoblastoma tumors with a defined *RB1* mutation, further mutational events are needed for malignancy (Corson and Gallie 2007). Retinal cells homozygous for *RB1* loss can form the benign tumor, retinoma, but develop other genomic changes in the progression to malignancy (Dimaras et al. 2008). The spectrum of changes is characterized by relatively few mutated genes, especially compared to common adult epithelial cancers. The transcriptional corepressor *BCOR* (BCL6 corepressor) is the most commonly mutated gene in retinoblastoma after *RB1* (Zhang et al. 2012). However, retinoblastoma displays a distinct pattern of genomic gains and losses, notably 1q, 2p, and 6p gain, and 16q loss (Kooi et al. 2016b). Candidate progression genes with functional evidence in these regions include *MDM4* and *KIF14* on 1q, encoding the p53 regulator mouse double minute 4 and the oncogenic motor protein kinesin family member 14, respectively. *MYCN* is the most commonly amplified gene on 2p, while on 6p, the best-studied genes are the DNA-binding proto-oncogene *DEK* and the transcription factor *E2F3*. Genes commonly lost on 16q include *CDH11*, encoding the adhesion protein cadherin-11, and *RBL2*, encoding the retinoblastoma family member p130 (Kooi et al. 2016b; Theriault et al. 2014). In addition, aberrant methylation

is seen in the retinoblastoma genome (Benavente and Dyer 2015), including of the oncogenic kinase *SYK* (spleen tyrosine kinase). As in other cancers, a variety of microRNAs are also dysregulated in retinoblastoma (Singh et al. 2016), including the miR-17~92 cluster and miR-106b~25 (Conkrite et al. 2011).

Treatment and prognosis. Treatment of retinoblastoma is complex and varied. Eyes containing tumors that display clinical features threatening to extend beyond the globe may be surgically removed (enucleation) and histologically assessed to determine risk of metastasis. Eye salvage may be attempted on less severely affected eyes (Dimaras et al. 2015; National Retinoblastoma Strategy 2009), with focal therapy (laser, cryotherapy) (Hamel et al. 2000), brachytherapy (Hernandez et al. 1993), and/or chemotherapy. Chemotherapy may be systemic, usually with a cocktail of vincristine, etoposide, and carboplatin (Chan et al. 1996; Gallie et al. 1996), intra-arterial (Yousef et al. 2016), and/or intravitreal (Munier et al. 2012a; Munier et al. 2012b)). Melphalan is the preferred drug for these latter modalities. External beam radiation is another effective treatment, however this is not preferred due to its added second cancer risks (Temming et al. 2016). Trilateral retinoblastoma treatment and outcomes are discussed in a later section.

Left untreated, retinoblastoma will grow and extend beyond the eye. Commonly, it invades the regional lymph nodes, bone, bone marrow, and the central nervous system. Extension into the CNS can be detected with radiologic imaging. Prognosis for CNS disease is much worse than for bone-marrow, as treatment with systemic chemotherapy is impeded by the blood-brain barrier (Mallipatna et al. 2017). Cytologic examination can detect tumor dissemination in CSF and bone marrow. Monitoring of CSF disease can be

also done molecularly, by looking for tumor-specific (non-germline) *RB1* mutations or post-*RB1* loss genomic gains and losses (Bowles et al. 2007; Dimaras et al. 2010b; Racher et al. 2016), or tumor-derived *GD2* or *CRX* mRNA expression (Laurent et al. 2010; Laurent et al. 2013; Torbidoni et al. 2015).

Individuals with a constitutional *RB1* mutation have an increased risk of developing second primary tumors, like osteosarcoma, melanoma, lung or bladder cancer later in life (MacCarthy et al. 2013). Surveillance for second primary malignancies is not yet supported by strong evidence or broad consensus, though some suggest there may be a role for annual screening by whole body MRI (Friedman et al. 2014; Kamihara et al. 2017). More commonly, retinoblastoma survivors are educated on second primary tumor risks and instructed to be vigilant about potential signs of new malignancies (Kamihara et al. 2017).

Guidelines for treatment in high income (National Retinoblastoma Strategy 2009) and low-and-middle income countries (Kenyan National Retinoblastoma Strategy Group 2014) are similar, although they account for differences in availability of resources and stage of disease at presentation (*i.e.*, low-and-middle-income countries see more cases of extraocular and metastatic disease). Patient outcomes are notably worse in low-and-middle income countries than in high (Dimaras et al. 2015), and further research is needed to address social determinants affecting access to quality care (Dimaras et al. 2010a).

IMAGING RETINOBLASTOMA

Clinical vignette. An adult survivor of bilateral retinoblastoma is pregnant with her first child. She undergoes prenatal genetic testing. Her fetus is found to carry her familial *RB1* mutation and is therefore at risk of developing retinoblastoma. Shortly after birth, the infant's eyes are visually examined, and the retina appears normal in each eye. Monthly examinations are scheduled, during which the ophthalmologist inspects each eye with the indirect ophthalmoscope, and an imaging specialist documents the fundus with digital images. At the second examination after birth, the ophthalmologist still does not see any tumors in the eyes. A clear fundus is documented in each eye using a high-resolution fundus camera. Next, the imaging specialist uses Optical Coherence Tomography (OCT) to image the eyes. Surprisingly, the OCT reveals a tiny lump within the inner nuclear layer of the retina in the left eye. Looking back at the fundus images, the ophthalmologist notes a slight haze in the exact spot picked up by OCT: it is a tiny tumor, originally missed on examination of the photographic images. The tumor is treated with focal therapy, preventing its growth and sparing vision (Based on similar cases previously reported; Rootman et al. 2013; Soliman et al. 2016).

Fundus imaging. Images of the fundus (interior surface of the eye), which become part of the medical record, facilitate consultation and collaboration, and precise tracking of tumor response or resistance to treatment. This kind of longitudinal, visual documentation is unique amongst CNS tumors. Before photographic imaging equipment was a standard part of ophthalmic care, retinal drawings (Wilson 1975) were used to map retinal topography, lesions, relevant landmarks and treatments applied, as observed through the indirect ophthalmoscope (Dvorak and Russell 2011). Retinal imaging has changed practice by providing a way to digitally document the eye, but

retinal drawings still remain an important part of learning to understand the disease trajectory.

Digital retinal imaging via portable fundus camera has revolutionized clinical documentation of retinoblastoma (Fig. 1b, 3a, 3d), allowing comparison of pre- and post-treatment images (Romanowska Dixon and Morawski 2017) and facilitating consultation between centers (National Retinoblastoma Strategy 2009). The RetCamTM (Clarity Medical) captures high-resolution, wide-angle photographs, and with scleral depression, allows imaging of the retina up to the *ora serrata* (the anterior edge of the retina) (National Retinoblastoma Strategy 2009). Similarly, the ICON camera (Phoenix Technology Group), produces wide-field, high-contrast, high-resolution fundus images.

Unlike fundus drawings, which depict the entire retina in one image, fundus images are partial views of the entire retina and must be stitched together to get a whole-retina view. Fluorescein angiography with portable fundus camera imaging is also important to document vascularization, especially for follow-up of advanced tumors (Kim et al. 2014).

Ultrasound. The B-scan (10 MHz) 2D ultrasound is commonly used for ocular imaging, and has been a part of the diagnostic toolkit in ophthalmology for decades (Giglio and Sherman 1979; Kendall et al. 2015; Mrochuk 1990). It is able to distinguish between ocular structures such as lens, choroid, retina and sclera, and image and measure aberrations in the eye, such as tumors or retinal detachment. Importantly for retinoblastoma, B-scan ultrasound allows visualization of calcification, a key feature of these tumors, and thus can help confirm diagnosis (National Retinoblastoma Strategy 2009; Novotny and Krasny 1990) (Fig. 3b).

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High frequency B-scan ultrasonography (20-50MHz), also known as ultrasound biomicroscopy (UBM), produces images with higher resolution than traditional B-scan (Pavlin et al. 1990). It first showed promise in the imaging of anterior segment tumors more consistently than B-scan (Pavlin et al. 1992; Reminick et al. 1998). For retinoblastoma, UBM has been shown to be essential for determining the proximity of the tumor to the anterior portion of eye (Vasquez et al. 2011). These are important diagnostic details that assist with accurate classification at presentation.

Furthermore, like other imaging modalities, ocular ultrasonography also allows monitoring of tumor regression as treatment progresses, primarily by comparing the size of the tumor at each examination to measurements taken at baseline. Unlike the use of intraoperative ultrasound for detection of residual disease in brain tumors (Bohringer et al. 2009), ocular ultrasound cannot currently distinguish between active and inactive retinoblastoma tumor.

Magnetic Resonance Imaging (MRI). As with other CNS tumors, MRI can provide important diagnostic and prognostic information in retinoblastoma (Fig. 3c) and is vital for screening for trilateral retinoblastoma (see below). MRI can also aid in characterizing the tumor in cases where it is not visible, for instance if it is behind a cataract-containing lens.

Where available, MRI is often used as part of the workup of all patients with retinoblastoma, as it can provide vital information about high-risk pathological features prior to enucleation (de Graaf et al. 2012). These include: optic nerve invasion evidenced by thickening; massive choroidal invasion evidenced by thickening or

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3 irregularities, scleral invasion evidenced by signal-enhancing tissue beyond the choroid,
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5 and intracranial invasion into the suprasellar region (Razek and Elkhamary 2011).
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7 However, sensitivity for detecting these high-risk features varies considerably (de Jong
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9 et al. 2014a). High field-strength MRI can also confirm retinoblastoma diagnosis by
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11 corroborating the calcifications observed by ultrasound or fundus imaging (Galluzzi et
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13 al. 2009), and can detect vitreous seeds or anterior segment involvement (Razek and
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15 Elkhamary 2011). Again, these uses are particularly valuable when fundus imaging is
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17 not possible. Note that computerized tomography (CT) imaging is generally avoided in
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19 retinoblastoma due to the radiation risks, especially in patients with a germline *RB1*
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21 mutation, already at increased risk for second primary tumors.
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27 In intracranial tumors like gliomas, intraoperative MRI has assumed a pivotal role, to
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29 detect removal of diseased tissue during surgery (Hlavac et al. 2017). Intraoperative
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31 MRI is not necessary in retinoblastoma, as enucleation usually removes the entirety of
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33 the tumor, unless extraocular spread has been previously detected, or high risk features
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35 are subsequently discovered on pathology. However, one retinoblastoma case report
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37 describes the use of intraoperative MRI to track the delivery and tumor penetration of
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39 intra-arterial melphalan delivered in a cocktail with gadolinium (Materin et al. 2012).
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44 **Optical coherence tomography (OCT).** OCT, a non-invasive imaging technique
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46 based on differential near-infrared light reflection, offers a powerful tool for imaging
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48 retinoblastoma, with resolution exceeding that of ultrasound or MRI. Conceptually
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50 similar to ultrasound, spectral domain (SD) OCT can generate high resolution, cross-
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52 sectional images of tissues where light can penetrate (Fujimoto and Swanson 2016), so
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54 has found utility in dermatology, urology, and in particular, ophthalmology, where it is
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3 routinely used in adult clinics. It has also been used in the CNS: for *ex vivo* analysis of
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8 trialed for intraoperative imaging of gliomas to detect residual tumor, as is also done via
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10 ultrasound and MRI (Bohringer et al. 2009).
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13 Recently, with the advent of hand-held intraoperative OCT systems, OCT has
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15 become popular for pediatric ophthalmology as well, including retinoblastoma
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17 assessment (Do et al. 2017). Initial publications were case reports indicating the value
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19 of OCT for detecting cavitory retinoblastoma (Mashayekhi et al. 2005) and reasons for
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21 visual loss (such as retinal detachment) post-therapy (Shields et al. 2005).
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23 Subsequently, OCT showed utility for documenting response to therapy, spotting edge
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25 recurrence, and notably, detecting “invisible” tiny retinoblastoma (Fig. 3d-f) (Rootman et
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27 al. 2013), as well as assessing foveal anatomy, which impacts the likelihood of visual
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29 function post-therapy (Cao et al. 2014; Samara et al. 2015). One case report even
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31 documented optic nerve head disease, usually detected by MRI (see above) (Yousef et
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33 al. 2012). OCT’s ability to look “behind” small features such as vitreous seeds to the
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35 underlying tumor or normal retina can be valuable for assessing retinoblastoma
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37 comprehensively.
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44 This growing body of work also contributed to an ongoing debate on the cell of origin
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46 of retinoblastoma, as some tiny tumors detected by OCT seem to originate in the inner
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48 nuclear layer (Rootman et al. 2013) (Fig. 3f), while others are more localized to the
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50 outer nuclear layer, suggestive of a cone precursor origin for at least some
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52 retinoblastoma (Berry et al. 2016), which is consistent with experimental data (Xu et al.
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54 2014).
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Although not yet in use for retinoblastoma, recently-developed OCT angiography holds considerable promise for documenting vascularization and blood flow in and around tumors (Gao et al. 2016). However, OCT does have limitations: it images the periphery of the retina poorly (although technology for widefield OCT is improving), and relies on transparent media: large tumors and/or cataracts limit its usefulness. Despite this, OCT for retinoblastoma is usually successful and can guide treatment decisions (Soliman et al. 2017b). With time, OCT will doubtless assume a greater role in the staging and management of retinoblastoma, taking true advantage of the accessibility of the eye to manage this cancer.

Imaging animal models. The wealth of imaging tools available for the eye has made longitudinal studies of retinoblastoma animal models possible, complementing more traditional histological analyses (Fig. 3g). The advent of small animal intraocular imagers enabled documentation of intraocular tumors in both transgenic and orthotopic xenograft models of the cancer. In particular, coupling fluorescence and brightfield imaging of GFP-expressing xenografts allowed qualitative assessment of tumor growth and (importantly) dissemination within the eye (Corson et al. 2014). Similarly, a number of preclinical therapeutic studies have included qualitative fundus and fluorescein angiography images (Brennan et al. 2011; McEvoy et al. 2011; Zhang et al. 2012). In the future, quantification of fluorescence should allow volumetric analysis of tumor growth, as is currently done using bioluminescence of luciferase-expressing xenografts (Corson et al. 2014; Ji et al. 2009; Laurie et al. 2005).

MRI has also been employed in preclinical treatment trials, and in one report was used for volumetric estimation of tumor (McEvoy et al. 2011). Ultrasound has also been

used for animal models in limited cases (Brennan et al. 2011); one study showed fundus, ultrasound and MRI images of transgenic animals in a treatment trial, but did not use these images for assessing efficacy (Nemeth et al. 2011).

OCT has become a favored method for detecting lesions, especially in genetic models. The first work in this area was done with custom-built OCT apparatus, enabling detection of intraretinal tumors in the SV40 T-antigen driven mouse model of retinoblastoma (TA_g-RB) (Ruggeri et al. 2007), and assessing their response to therapy (Cebulla et al. 2008). Even retinal tumors *in utero* could be detected through the uterine horn (Larina et al. 2012). TA_g-RB tumors have also been documented by ultrasound (Foster and Brown 2012).

More recently, using a commercially-available small animal OCT system, we showed that this technique can detect the earliest signs of tumors in the TA_g-RB model, corresponding to their first appearance by histology (Fig. 3g) (Wenzel et al. 2015), and we used this finding to show that overexpression of the oncogene *Kif14* accelerates not just tumor growth, but tumor initiation in this model as well (O'Hare et al. 2016).

OCT has proven useful for xenografts: intravitreal xenografts could be monitored (Corson et al. 2014; Tschulakow et al. 2016) and subretinal xenografts could be rapidly assessed by a combination of OCT and funduscopy (Lemaitre et al. 2017). Of course, OCT assessment of morphology of xenografts is less clinically relevant than the morphology of tumors arising intraretinally as in genetic retinoblastoma models. However, the prospects for rapidly assessing genetic modifiers and/or therapeutic efficacy by OCT in both types of model are appealing, especially with the advent of

methods to volumetrically assess lesions on OCT (Ruggeri et al. 2009; Sulaiman et al. 2015).

MYCN-AMPLIFIED RETINOBLASTOMA

Clinical vignette. A 1-month-old infant with no family history of retinoblastoma has a large retinoblastoma identified in one eye. On enucleation, no rosettes are noted, but the high-risk pathological feature of optic nerve invasion is present. Since early-onset, aggressive tumors are often indicative of a germline *RB1* mutation, heritable disease is suspected, with the concomitant risk of tumors in the fellow eye and a need for lifelong surveillance and risk to offspring. But genetic analysis of the enucleated tumor fails to find any mutated *RB1* alleles, despite >96% detection rate in specialized labs (Soliman et al. 2017a). However, further analysis of common copy number changes in retinoblastoma reveals that the oncogene *MYCN* is amplified to 53 copies in this tumor, and on subsequent re-review, the pathologist notices some neuroblastoma-like histological features. These findings strongly suggest *MYCN*-driven disease, which is not heritable, leaving this child at only population risk for future malignancies.

Discovery and characteristics. *MYCN* retinoblastoma was identified based on careful analysis of over 1000 tumor genotypes (Rushlow et al. 2013). Although *MYCN* amplification had been documented in occasional retinoblastoma since the *MYCN* gene was first identified (Gilbert et al. 1981), this large-scale study was needed to reveal that *MYCN* amplification is much more common amongst unilateral retinoblastoma without a detectable *RB1* mutation. This finding has since been replicated in other cohorts (Ewens et al. 2017; Kooi et al. 2016a; McEvoy et al. 2014), but it is important to note that *MYCN* retinoblastoma is currently only identifiable after enucleation. Retrospective

analysis of *MYCN* retinoblastoma revealed several further differentiating features (Fig. 4). Clinically, these tumors are amongst the very earliest-onset unilateral tumors, and often had high-risk pathological features, although there is not yet sufficient evidence to conclude that *MYCN* amplification has a dramatic effect on aggressiveness, as it does in neuroblastoma or medulloblastoma (Ruiz-Perez et al. 2017). Histopathologically, these tumors also differ from *RB1*^{-/-} retinoblastoma. Comparatively, they show more rounded, undifferentiated cells, with prominent nucleoli (Fig. 4c), and lack the nuclear molding, differentiated rosettes, and extensive calcification commonly seen in retinoblastoma. Again, the histology of *MYCN* retinoblastoma is more similar to neuroblastoma than it is to *RB1*^{-/-} retinoblastoma.

Molecular features. Beyond the lack of *RB1* mutations, *MYCN* retinoblastoma had fewer copy number alterations than *RB1*^{-/-} tumors in one study (Rushlow et al. 2013), although this was not seen in a subsequent report (Ewens et al. 2017). *MYCN* retinoblastoma were also less likely to display the characteristic chromosomal gains and losses of retinoblastoma (1q and 6p gain, 16q loss), instead showing some variations in common with neuroblastoma (17q gain, 11q loss) (Rushlow et al. 2013).

MYCN retinoblastomas have functional, hyperphosphorylated pRB (Ewens et al. 2017), and high levels of N-Myc protein (Rushlow et al. 2013). The fact that pRB is expressed, yet inactivated by post-translational modification, strongly implies that genetic changes at the *RB1* locus are not driving this disease. The SKP2-p27 axis leading to pRB phosphorylation was not activated in 3 of 4 *MYCN* retinoblastoma (Ewens et al. 2017), suggesting that this *MYCN*-activated pathway is not a key driver in *MYCN* retinoblastoma, unlike in neuroblastoma (Evans et al. 2015).

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3 Interestingly, high retinal *Mycn* levels alone (driven by the Pax6 α enhancer) could
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5 not initiate retinoblastoma-like tumors in mice, but could cooperate with *Rb1* loss to form
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7 tumors (which normally require loss of another retinoblastoma family member such as
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9 *p107*) (Wu et al. 2017). This discovery also offered a caution toward *MYCN*-targeted
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11 therapy: when *Mycn* levels were reduced in this mouse model, tumors reoccurred with
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13 *Mycn*-independence (Wu et al. 2017). Further insight into how *MYCN* amplification
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15 leads to pRB inactivation by hyperphosphorylation and drives the human tumors is
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17 needed. Perhaps the Pax6 α enhancer does not drive expression in the retinal cell type
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19 susceptible to *Mycn*-driven retinoblastoma; *Mycn* overexpression alone can promote
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21 cancer in other tissues, as observed with the tyrosine hydroxylase-driven *Mycn*
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23 neuroblastoma model (Weiss et al. 1997). In the absence of perfect mouse models, cell
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25 lines and patient-derived xenografts will be best suited for these experiments, but given
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27 the rarity of *MYCN* retinoblastoma, few are currently available.
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34 **Are *MYCN*-amplified ocular tumors retinoblastoma?** Despite the
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36 histopathological and genetic similarities with neuroblastoma, there is no reason to
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38 believe that “*MYCN* retinoblastoma” is not retinoblastoma: it apparently arises in a
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40 retinal neuronal precursor, and (based on a small number of tumors) has a gene
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42 expression profile not statistically distinct from that of a major group of retinoblastoma,
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44 characterized by low “photoreceptoriness”, but high expression of RNA biogenesis and
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46 M phase genes (Kooi et al. 2015). Thus, to date it is unclear if *MYCN* retinoblastoma
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48 derives from a different retinal cell of origin than *RB1*^{-/-} retinoblastoma, or if the cell of
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50 origin is the same, but the initiating *MYCN* amplification event pushes these cells into a
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52 distinct phenotype.
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TRILATERAL RETINOBLASTOMA

Clinical vignette. A 6-month old presents with a 2-week history of leukocoria in one eye. Upon ophthalmic examination, the child is diagnosed with unilateral retinoblastoma. As part of routine work-up, the child undergoes CT of head and both orbits. The CT reveals an intracranial midline tumor. Cytologic examination of the CSF shows presence of malignant cells. The child undergoes treatment for trilateral retinoblastoma, which includes high-dose chemotherapy followed by autologous stem cell transplant. The child is alive and well 10 years post-treatment. (Based on a previously reported case; Dimaras et al. 2015; Dimaras et al. 2011).

A 4-month old presents to the emergency room with atypical eye movements and overgrowth syndrome. A CT scan reveals a suprasellar tumor (Fig. 5). The eyes are not examined at this time. The tumor is biopsied and shows a small blue cell tumor with abundant Flexner-Wintersteiner rosettes. The eyes are then examined, and reveal multifocal tumors in both eyes. Systemic and intrathecal chemotherapy for trilateral retinoblastoma is prescribed. During the placement of the intrathecal catheter, the treating physicians observe tumor seeding along the needle tract of the initial biopsy. The child's tumor reoccurs and she dies. (Based on a previously reported case; (Dai et al. 2008; Dimaras et al. 2011).

Origins and Pathology. Trilateral retinoblastoma, first described in 1977 (Jakobiec et al. 1977) and coined in 1980 (Bader et al. 1980), is the heritable form of retinoblastoma associated with an intracranial tumor. The intracranial tumors most commonly affect the pineal gland, as well as the suprasellar or intrasellar region of the brain (Fig. 5). Pineal tumors are generally smaller than non-pineal tumors in trilateral

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3 retinoblastoma (de Jong et al. 2014b). Benign pineal cysts have also been observed
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5 with retinoblastoma (Beck Popovic et al. 2006; Gupta et al. 2016; Karatza et al. 2006).
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7 In rare cases pineal cysts can transform into malignant tumors, suggesting they should
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9 be monitored over time (de Jong et al. 2016). Cystic normal pineal glands, however, are
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11 also regularly observed in children; their size is often comparable to solid and cystic
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13 pineoblastoma (Sirin et al. 2016). This makes it challenging to know which cystic lesions
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15 require follow-up, and which do not. A classification for pineal glands has been
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17 proposed to differentiate glands based on absence or presence of cysts, number of
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19 cysts, and enlargement (Type 0-4) (Sirin et al. 2016). The European Retinoblastoma
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21 Imaging Collaboration proposed guidelines for follow-up of cystic pineal lesions based
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23 on this classification: they recommend monitoring Type 3 and 4 lesions (multicystic with
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25 enlargement) by routine MRI for changes in size or morphology of the solid component
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27 of the lesion (Sirin et al. 2016). It is sometimes equally challenging to differentiate large
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29 solid pineal glands from pineoblastoma (Galluzzi et al. 2016).
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36 Trilateral retinoblastoma often presents with leptomeningeal metastasis or CSF
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38 involvement, which is difficult to cure. The histological features of the intracranial tumor
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40 are identical to those of retinoblastoma (Fig. 5c). Because of its rarity, little work on the
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42 molecular genetics of this tumor has been reported (Li et al. 2005). Mouse models may
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44 yet offer some insights: 15%-27% of TAg-RB mice develop primitive neuroectodermal
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46 tumors, analogous to the parasellar and suprasellar tumors arising in patients with
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48 trilateral retinoblastoma (Marcus et al. 1991; O'Brien et al. 1990). The trilateral tumors in
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50 mice have histological features consistent with their human counterparts (Marcus et al.
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55 1991). However, this murine retinoblastoma model does not develop pineoblastoma
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(Marcus et al. 1991), although other T-antigen-driven tumor models do (Marcus et al. 1996). Also, *Rb1*^{+/-} mice with loss of p53 develop pineoblastomas, but these mice do not develop retinal tumors (Williams et al. 1994). Despite the existence of these models, no preclinical studies on trilateral retinoblastoma have yet been reported.

In addition to predisposition by *RB1* mutation, pineoblastoma alone is associated with germline mutations in *DICER1* (de Kock et al. 2014). In murine retinoblastoma models, *Dicer1* is a haploinsufficient tumor suppressor (Lambertz et al. 2010). Further investigation is warranted into the development of pineoblastoma within and without the context of retinoblastoma.

Epidemiology. The incidence of trilateral retinoblastoma among patients with heritable retinoblastoma is 3.5% (de Jong et al. 2015). Prior systemic chemotherapy to treat retinoblastoma is associated with decreased risk of developing an intracranial tumor (Shields et al. 2001). Suprasellar or intrasellar tumors are diagnosed earlier than pineal, and often concurrently with the retinoblastoma (Kivela 1999). In contrast, 60% of pineal tumors are diagnosed within 1 year of initial retinoblastoma diagnosis, suggesting that routine screening by MRI could contribute to earlier diagnosis (de Jong et al. 2014b). Some centers screen by MRI every 6 months until 5 years of age (Kamihara et al. 2017), however it is unclear if this approach is warranted or effective (De Ioris et al. 2014).

Becoming treatable. Treatment for trilateral retinoblastoma generally involves chemotherapy delivered systemically and intrathecally via an Ommaya reservoir, followed by high-dose chemotherapy and autologous stem cell rescue (Dimaras et al.

2011). Surgical treatment has been attempted in some cases; however, complete resection is difficult due to the locations of the intracranial tumors (Dunkel et al. 2010). Craniospinal radiation, while effective, is not preferred because of its long-term developmental, neurocognitive and endocrine damage (Duffner et al. 1985; Mulhern et al. 1999). Indeed, the use of radiation in the treatment of trilateral retinoblastoma has declined in recent years (de Jong et al. 2014b).

Survival from trilateral retinoblastoma has improved over time, most notably with the introduction of high-dose chemotherapy and autologous stem cell transplant (de Jong et al. 2014b). Looking at outcomes before and after 1995, when chemotherapy changed treatment for retinoblastoma, the 5-year survival for pineal trilateral retinoblastoma has increased from 6% to 44%, and non-pineal from 0 to 57% (de Jong et al. 2014b; Kivela 1999). The presence of leptomeningeal metastases considerably reduces probability of survival (de Jong et al. 2014b).

CONCLUSIONS AND OUTLOOK

Although rarer than many other CNS cancers, retinoblastoma has offered fundamental cancer knowledge, and clinicians and researchers have taken advantage of its accessibility to carefully document and treat it using multiple modalities. We envisage several areas for future developments to improve our understanding and clinical management of this cancer.

Trilateral retinoblastoma. Our improving knowledge of the clinical behavior of intracranial tumors in retinoblastoma has not been matched by our understanding of their molecular features. It will be valuable to determine if there are molecular

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characteristics of certain heritable retinoblastomas that might predispose to formation of
trilateral tumors. These might include *RB1* genotype, *DICER1* genotype, protein marker
expression, features on high resolution imaging, or others. Trilateral retinoblastoma
tumors (like their anatomical counterparts not associated with retinoblastoma) might
benefit from targeted therapy, and each could benefit from discoveries in the other in
this domain. The availability of mice that develop trilateral retinoblastoma-like tumors
makes preclinical studies technically feasible, but the low frequency of intracranial tumor
formation in these mice plus the need for histological diagnosis makes such studies
challenging.

MYCN retinoblastoma. The study of *MYCN* retinoblastoma can gain much from the
wealth of knowledge available for *MYCN* neuroblastoma (Huang and Weiss 2013).
Despite caveats (Wu et al. 2017), the potential for targeted agents borrowed from
neuroblastoma research to treat *MYCN* retinoblastoma is exciting. Currently, these
would only be useful for metastatic disease as the *MYCN* genotype is only determined
post-enucleation (recall that intraocular retinoblastoma is not biopsied due to risk of
tumor dissemination). But a promising new ‘liquid biopsy’ technique could change this,
as it allows for capture and molecular analysis of tumor DNA from the aqueous humor
of the eye, which can be safely removed with techniques that minimize risk of tumor
spread (Berry et al. 2017). This approach could potentially be used to identify *MYCN*
tumors *in situ*. Moreover, if distinguishing characteristics of *MYCN* tumors could be
discovered in OCT, fundus, ultrasound, or magnetic resonance imaging, or in circulating
tumor cells or DNA, targeting the primary tumor would also be possible. Large,
prospective studies will be needed to find these characteristics. Similarly, comparison of

the molecular features (genomic, proteomic, and signaling changes) of *MYCN* retinoblastoma with *MYCN* neuroblastoma may reveal shared vulnerabilities or unique characteristics.

Deciphering the cell of origin of *MYCN* retinoblastoma is likewise important. One way to explore this would be to parallel the work that identified cone precursors as a likely origin of at least some *RB1*^{-/-} retinoblastoma by knocking down *RB1* in dissociated human fetal retinal cells and observing which cell types can proliferate (Xu et al. 2014). A similar experiment, overexpressing *MYCN* instead of removing *RB1*, could perhaps provide insight into retinal cells susceptible to proliferation after *MYCN* gain.

Imaging. Tumor imaging has revolutionized care of retinoblastoma, and could perhaps be further applied to research on other CNS tumors. The powerful combination of fundus imaging and OCT makes the prospect of xenografting other tumor types into the eye an appealing one, especially for neural tumors where intravitreal or subretinal injection would be more facile than intracranial xenografting. Although the intraocular microenvironment differs from the brain, it offers similarities (neuronal and glial neighboring cells, separation from the blood by a semi-permeable barrier) that subcutaneous xenografts lack. Such paratopic xenografts could be easily followed using the imaging modalities above, and will be an interesting area for further exploration.

Retinoblastoma may also learn from imaging in CNS tumors. Molecular imaging via positron emission tomography (PET) or single photon emission computed tomography (SPECT) is showing promise for brain tumors, especially using radiolabeled amino acid tracers (Brindle et al. 2017). But the rare research on these imaging modalities in

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retinoblastoma has shown little-to-no promise, offering no additional insight on tumor features or response to treatment (Moll et al. 2004). Further research including novel tracer development may be indicated. However, the need to keep radiation exposure low for heritable retinoblastoma cases must also be considered.

Large imaging studies might also reveal other imaging biomarkers that could help stratify retinoblastomas for improved prognostication and treatment decisions. Such work could impact identification of both trilateral-predisposed and *MYCN* retinoblastoma, as noted above. Further ability to look out for distinctive features in the eyes of retinoblastoma patients will continue to improve the outlook for this visible CNS tumor.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

ROLE OF AUTHORS

Conceptualization: H.D., T.W.C.; Writing – Original Draft: H.D., T.W.C.; Writing – Review & Editing: H.D., T.W.C.; Visualization: H.D., T.W.C.

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FIGURE LEGENDS

Fig. 1. Clinical features of retinoblastoma. (a) External RetcamTM image of retinoblastoma; calcified tumor is visible through the pupil as leukocoria. (b) RetcamTM fundus image revealing two tumors (arrowheads), * = optic nerve head. (c) Gross pathology of an enucleated eye. A large, calcified tumor is apparent on the left side of the globe (arrowhead). (d) Pupillary-optic nerve section of an eye enucleated for retinoblastoma as primary treatment. (e) Homer Wright rosettes (arrowhead) and packed cells; (f) Viable collars of cells around blood vessels, and abundant necroses between them; (g) Area of retinoma, with fleurettes (arrowhead). Scale bars = 100 μ m.

Fig. 2. Retinoblastoma genetics. The cancer can initiate with a germline *RB1* mutation followed by a single somatic hit, or with two somatic mutations (M1 and M2). Loss of *RB1* leads to the benign retinoma; further mutations (M3 to Mn) are required for malignancy. Retinoblastoma can also initiate by amplification of *MYCN*, with other genomic changes as yet unknown.

Fig. 3. Retinoblastoma imaging. (a-b) RetcamTM (a) image of an eye reveals two large tumors, and ultrasound image (b) allows measurement of tumor height (yellow and green lines). (c) Axial T1 MRI post-gadolinium reveals tumor at back of eye (arrowhead). (d-f) Imaging of the eye of an infant with heritable retinoblastoma by RetcamTM (d) shows no visible tumor. OCT scan line shown in green/red. OCT imaging (e, en face; f, transverse section) reveals small tumor appearing to arise out of the inner nuclear layer of the retina (arrowhead). (g) Fundus photo (top) and OCT image (bottom) show a retinoblastoma-like tumor (arrowheads) arising in the TAg-RB mouse model. OCT scan line shown in red. Scale bars = 100 μ m.

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Fig. 4. *MYCN* retinoblastoma. (a) Tumor appears retinoblastoma-like on fundus image. (b) Ultrasound reveals characteristic calcification. (c) Histopathology of tumor in enucleated eye reveals round nuclei with prominent large multiple nucleoli; contrast with *RB1*^{-/-} retinoblastoma in Fig. 1e. Adapted with permission from (Rushlow et al. 2013).

Fig. 5. Trilateral retinoblastoma. (a) Sagittal T1-weighted and (b) coronal T2-weighted fluid-attenuated inversion recovery (FLAIR) contrast MRI reveals suprasellar mass in a patient who also has bilateral retinoblastoma. (c) Histology of this mass reveals abundant Flexner-Wintersteiner and Homer Wright rosettes. Scale bar = 25 μm.

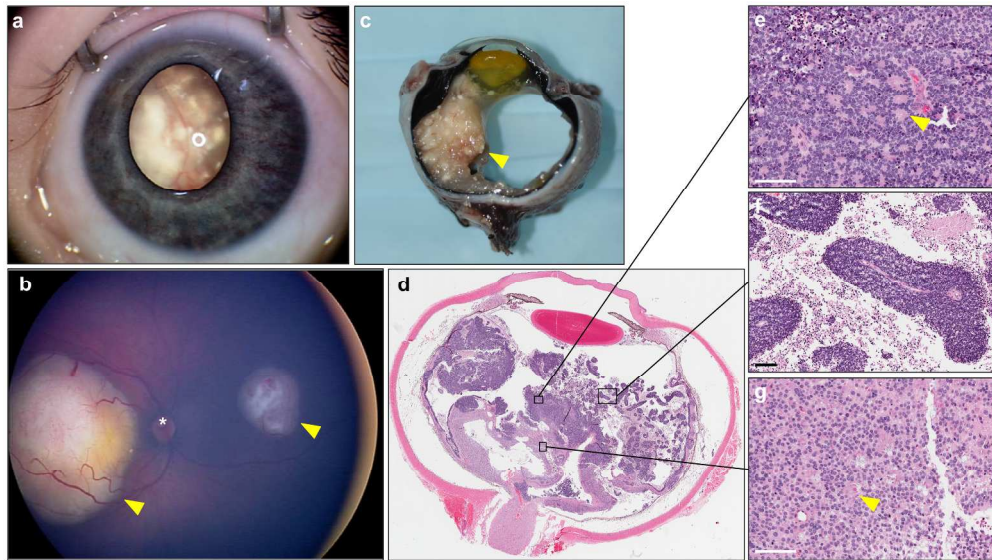


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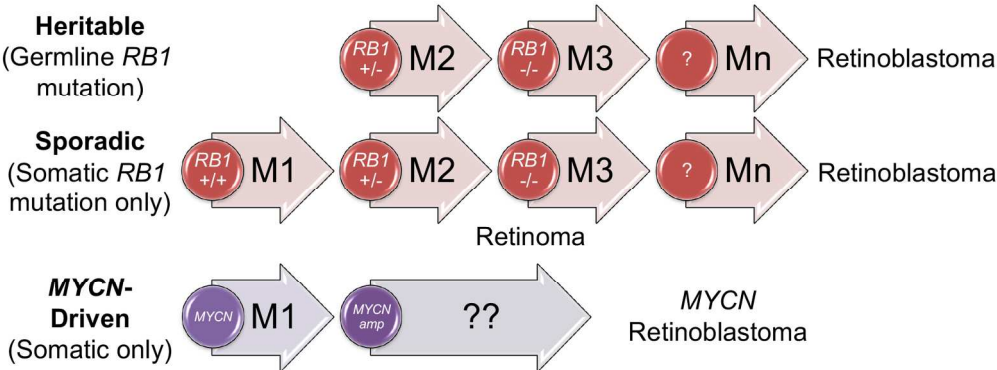


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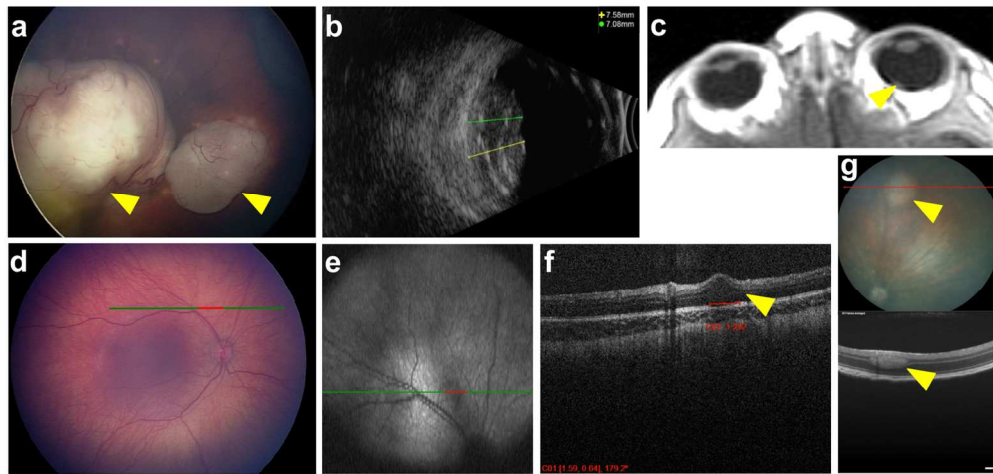


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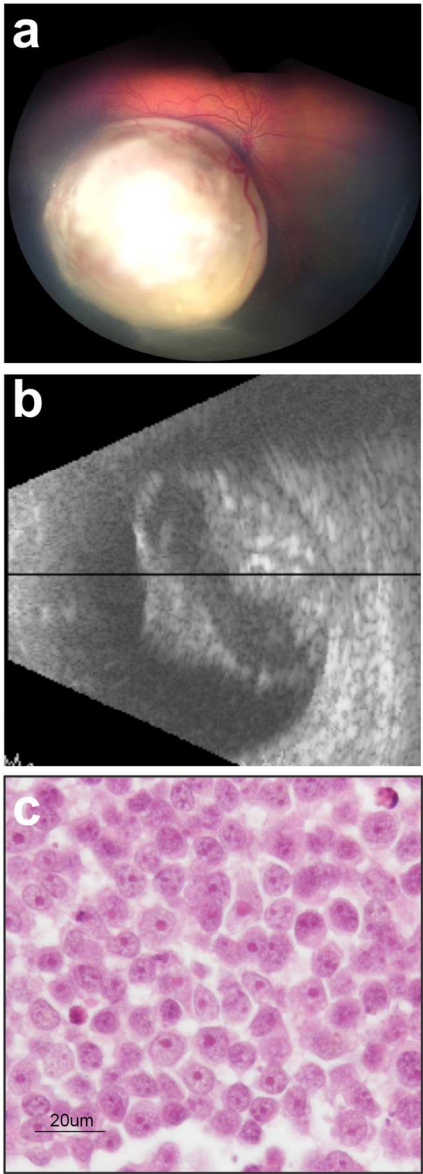


Fig. 4. MYCN retinoblastoma. (a) Tumor appears retinoblastoma-like on fundus image. (b) Ultrasound reveals characteristic calcification. (c) Histopathology of tumor in enucleated eye reveals round nuclei with prominent large multiple nucleoli; contrast with RB1-/- retinoblastoma in Fig. 1e. Adapted with permission from Rushlow et al. 2013.

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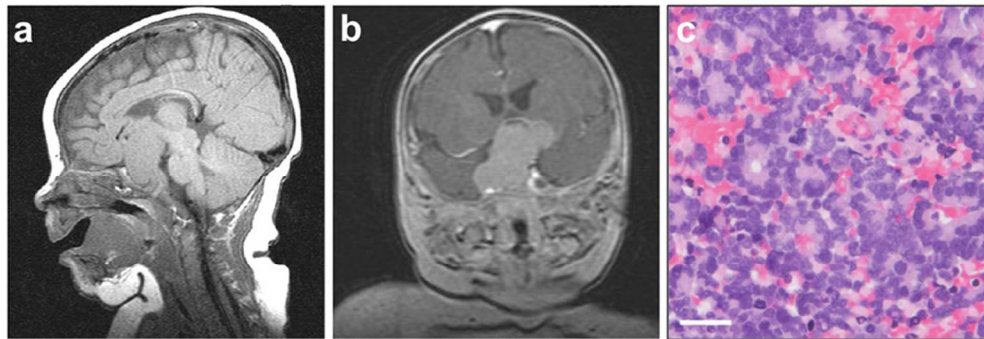
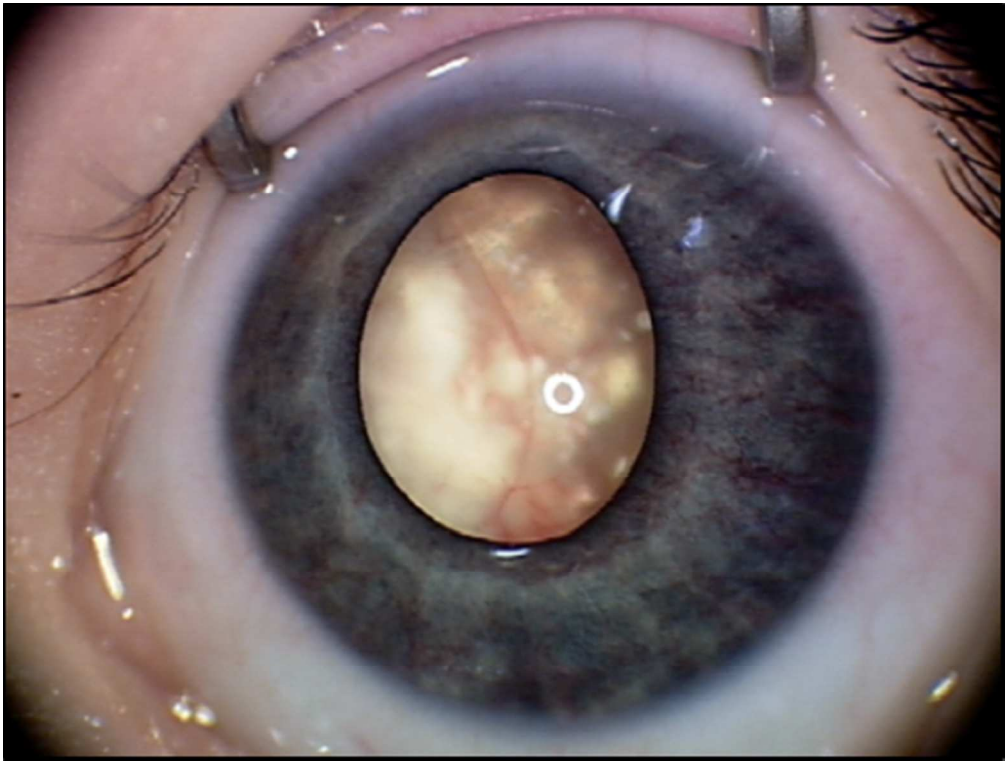


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81x28mm (300 x 300 DPI)



Retinoblastoma is a uniquely accessible CNS tumor. Imaging techniques for this cancer, the biology of its MYCN-driven form, and its “trilateral,” intracranial manifestation provide interesting contrasts and parallels with other CNS malignancies.

103x78mm (150 x 150 DPI)

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For Peer Review